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# Effect of cadmium chloride on liver, spleen and kidney melano macrophage centres in *Tilapia mossambica*

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***Abstract:*** *To study the toxic effect of a heavy metal on the occurrence of melano macrophage centres (MMC) of liver, spleen and kidney. Tilapia mossambica were exposed to median lethal concentration of cadmium chloride for 120 hours. Routine histological method was adopted to prepare the tissue sections and to identify the pigments viz: hemosiderin and melanin. The average number and size of melano macrophage centres (MMC) were significantly increased compared with the control. It is evident in the present study that in the MMC of all three tissues examined lipofuscin is absent.*

***Key words:*** *Cadmium chloride, Tilapia mossambica, Melano macrophage centres, Pigments, Lipofuscin*
*PDF of full length paper is available online*

## Introduction

Heavy metals from industries disturb the aquatic environment and leads to environmental health hazards (Shukla *et al.,* 2007; Gupta and Srivastava, 2006; Agtas *et al.,* 2007; Yoon *et al.,* 2008). Among the heavy metals, cadmium is considered as a major aquatic pollutant in many parts of the world. The two main arms of the immune response of the fish are innate and adaptive. The innate arm is the first line of defense against pathogens which act quickly and non-specific and lacks memory, whereas the adaptive arm is second line of defense which displays memory. Innate immunity is carried out by non-immunological and immunological protective mechanism. Phagocytosis, the cellular ingestion and digestion of a particulate matter, a defense mechanism is present in all animals. Phagocytosis is mediated by macrophages and polymorphonuclear leucocytes. In fishes pigment containing cells are a prominent feature of haemopoietic tissues. These cells are identified as macrophages, containing greenish brown pigment representing breakdown products of haemoglobin from degenerated red blood cells (Roberts, 1975). Three types of pigments are found under normal conditions, melanin, haemosiderin and lipofuschin. These three pigment types can be present in one and the same macrophage. There are several reports on the melano macrophage centres in a wide range of fish (Passantino *et al*., 2005a,b; Agius and Roberts, 2003; Herraez and Zapata, 1991; Pratap and Bonga, 2007).

The innate immune system is something common to all multicellular organisms. Many components of the innate immune system appear to the evolutionarily conserved (Ulevich, 2000; Hoffman *et al*., 1999). Bols *et al*. (2001) reviewed the toxicity induced immunodulation of fish innate immunity. There are several reports on the immunotoxic effect of cadmium and other heavy metals on several fish species in relation to humoral and cell mediated immune response. The role of macrophages and melanomacrophage centres and their pigments has been used as a biomarkers for environmental pollution by several authors, but the relationship between these structures and the endogenous factors is not completely explained (Rabitto *et al*., 2005). The present study is aimed to focus on the effect of heavy metal cadmium chloride on the occurrence of melano macrophage centres (MMC) of liver, spleen and kidney of *Tilapia mossambica*.

In the present study the concentration was chosen on the basis of acute toxicity test. The reason for taking one concentration is the study aimed to evaluate the effect of cadmium on MMC and not on the comparison based on concentrations.

## Materials and Methods

Mature *Tilapia mossambica* of both sexes were collected from the local fish ponds. Fishes weighing about 75-80 g with a body length ranging from 18-20 cm were acclimatized to laboratory conditions. Fish were treated with 2% $KMnO_4$ solution for 15 minutes to remove external contamination and the water was renewed every 24 hr prior to the toxic exposure in plastic aquarium tanks in dechlorinated tap water at temperature of 28 ± 1°C with 12 hr light and dark photoperiod. The tap water used in the experiments having the following physicochemical characteristics (APHA, 1989): dissolved oxygen, 7.0-8.0 ppm; salinity, 0.4-0.5 ppm; alkalinity, 250 mg $l^{-1}$ as $CaCO_3$; hardness, 370 mg $l^{-1}$ as $CaCO_3$ and pH, 7.4-7.8. The fishes were fed with commercial supplemented feed (Red Rose Ltd). Having fish meal, wheat flour, soybean meal, corn meal, yeast, vitamins and minerals (Composition: crude protein -32%, crude fat -4%, fiber -5%, moisture -10%, vitamins, minerals and others) provide by supplier.

The 120 hr median lethal concentration (120 hr $LC_{50}$) of cadmium chloride (E.Merck) was estimated following the method of Litchfield and Wilcoxon (1949).

For the experiments, two groups of fishes were maintained one served as a control and another one as test group. In each group 10 fishes were introduced into 200 liter

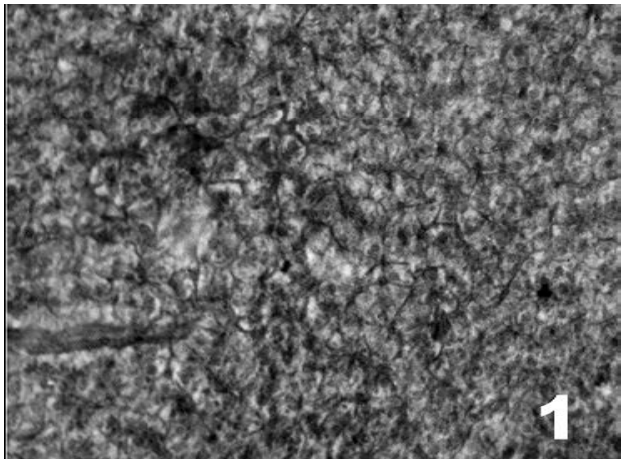


**Fig. 1:** Section of the liver of *T. mossambica* (control) H&E X 100

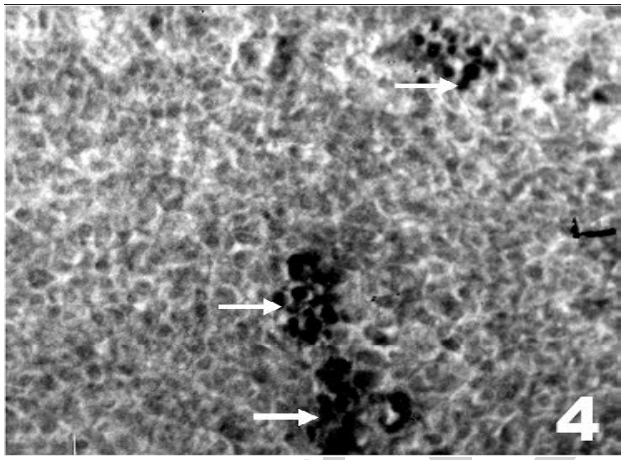


**Fig. 4:** Section of the liver of *T. mossambica* (treated) showing the melano macrophage centres (MMC) H&E X 200

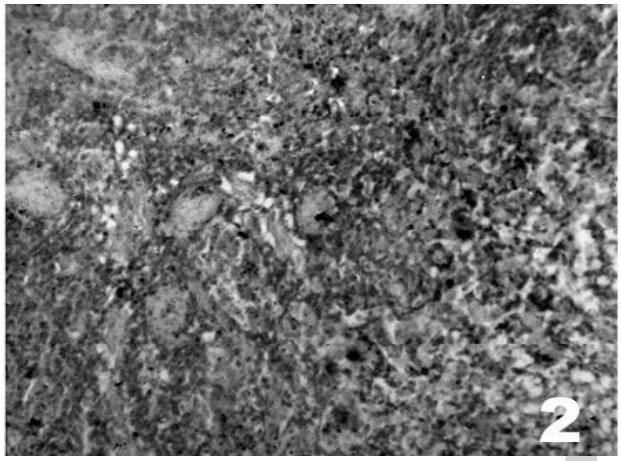


**Fig. 2:** Section of the spleen of *T. mossambica* (control) H&E X 200

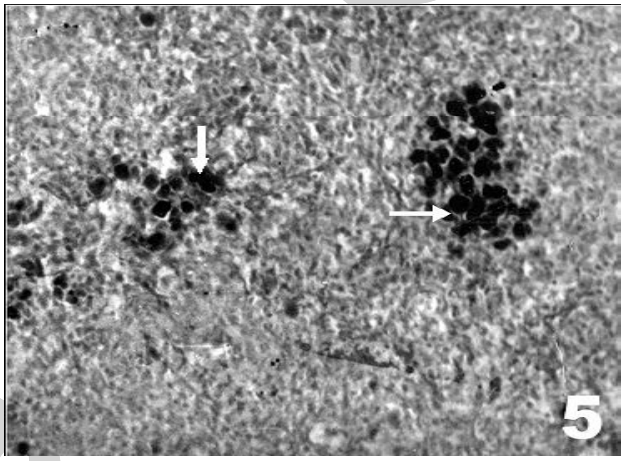


**Fig. 5:** Section of the spleen of *T. mossambica* (treated) showing the MMC H&E X 200

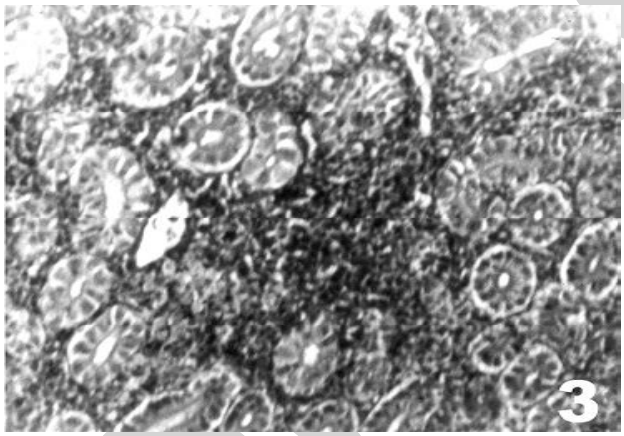


**Fig. 3:** Section of the kidney of *T. mossambica* (control) H&E X 400

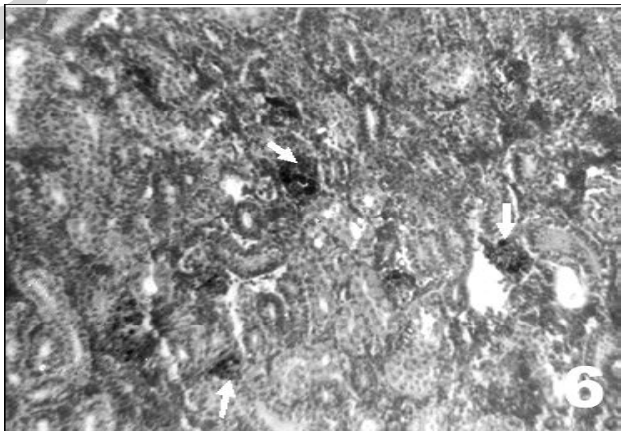


**Fig. 6:** Section of the kidney of *T. mossambica* (treated) showing the MMC H&E X 200

aquarium tanks. For the experiments as test group one tenth (20.93 mg $l^{-1}$) of the 120 hr $LC_{50}$ concentration was used.

**Histology:** For the histological preparation, liver, spleen and kidney tissues were dissected from the control and exposed fishes. The fishes were exposed for 120 hr (5 days). The tissues were fixed in Bouins fluid or Neutral buffered formalin for 24 hr. The fixed tissues were processed and sections were stained in Delafield haematoxylin and eosin.

**Hemosiderin :** Sections were deparaffinised, hydrated, immersed in yellow ammonium solution for one hour. The sections were washed in distilled water and immersed in 10% haematoxylin

**Table-1:** Changes in the number and size of melano macrophage centres (MMC) / organ during 120 hr exposure of cadmium chloride (20.93 mg $l^{-1}$) to *T. mossambica*

| Organ | Number of MMC /organ | | Size of the MMC (μm) | |
|---|---|---|---|---|
| | Control | Treated | Control | Treated |
| Liver | 7.25 ± 1.1 | 29.5 ± 2.12* | 2.3 ± 0.511 | 11.5 ± 2.5* |
| Spleen | 39.0 ± 1.4 | 64.5 ± 4.9* | 3.0 ± 0.617 | 15.0 ± 3.0* |
| Kidney | 11.25 ± 1.15 | 30.5 ± 2.5* | 6.3 ± 1.315 | 31.5 ± 6.5* |

Values are expressed as mean ± S.D. (n=5 fishes) significant at $p<0.05$

(alcoholic) for 1-4 hours. Then the sections were washed in 70%, absolute alcohol and followed by a rinse in 50% alcohol and washed in distilled water. Couterstained in neutral red. Mounted with DPX Hemosiderin appears as Dark blue.

**Lipofuscins :** The sections were deparaffinised, hydrated, immersed in 1% Ferric chloride (aqueous) and washed in running water, rinsed in 1% potassium hydroxide (alcoholic) followed by immediate rinse in 70% alcohol, washed in distilled water and counter stained in neutral red. Mounted with DPX. Lipofuscins appears as dark blue.

**Melanin :** Deparaffised the sections, hydrated, immersed in 2.5% ferrous sulphate solution for one hour, washed in distilled water for 20 minutes, immersed in 1% potassium ferricyanide for 30 minutes and washed in 1% acetic acid , mounted with DPX. Melanin appears as green. Differentiation of melanin and Lipofuscins.

**To differentiate melanin and lipofuscins** Nile blue method was adopted. The deparaffinised sections were hydrated, stained in Nile blue A solution for 20 min, washed in running water for 10-20 min and mounted with DPX. Lipofuscins appears as blue and melanin colourless.

The pigments hemosiderin, lipofuscin and melanin were identified by following the method as described by Gurr (1958). The histological examination of the tissue sections included counting of MMC per organ, planimetric measurements of MMC by following the method described by Kranz and Gercken (1987) size and organ areas were examined under Nikon-Trinocular microscope and micro-photographs were taken. Statistical analysis were performed by the Student's 't' test.

## Results and Discussion

The median lethal concentration ($LC_{50}$) value for 120 hr exposure of cadmium chloride was 209.34 mg $l^{-1}$. Exposure to cadmium chloride (20.93 mg $l^{-1}$) produced significant changes in melano macrophage centres (MMC) and free macrophages of liver, spleen and kidney of *Tilapia mossambica*.

In the control fish, liver contains few melano macrophage centres (MMC) and free macrophages. The MMC are smaller in size and irregular in shape and appeared as aggregate clusters near the blood vessels (Fig. 1), the size is 2.3 ± 0.511 μm and the average number per organ is 7.25 ± 1.1 (Table 1). In spleen, the free macrophages and melano macrophage centres distributed to the entire gland (Fig. 2), the size is 3.0 ± 0.617 μm and the average number per organ is 39 ± 1.4 (Table 1). In kidney the macrophages both free and MMC are present along with hematopoietic tissues (Fig. 3). The size of the MMC is 6.3 ± 1.315 μm and the average number per organ is 11.25 ± 1.115 and size of free macrophages is 2.5 ± 0.509 μm (Table 1).

In the liver of exposed fish the average number of MMC and free macrophages increases when compared with control. The size of MMC is 11.5 ± 2.5 μm and number of MMC per organ is 29.5 ± 2.12. In the spleen, size is 15 ± 3.0 μm and average number is 64.5 ± 4.9 per organ. In kidney increased number of free macrophages as well as MMC is noticed. The size of free macrophages is 12.5 ± 2.5 μm and MMC is 31.5 ± 6.5 μm. The average number of MMC per organ is 30.5 ± 2.5. There is a significant ($p<0.05$) statistical difference in the size and the frequency of MMC that exists between the control groups with the exposed fishes (Table 1).

**Pigments:** In both control and cadmium chloride treated fish the MMC appeared as yellow or dark brown in colour. Hemosiderin and melanin positive MMC is present in the liver, spleen and kidney. Lipofuscin pigment is absent in the liver, spleen and kidney of treated fishes.

In the present study there is a significant difference in the frequency and size of melano macrophage centres (MMC) and free macrophage found in liver, spleen and kidney of *Tilapia mossambica* exposed to 20.93 mg $l^{-1}$ of cadmium chloride. These findings are consistent with the earlier reports (Suresh and Veeraraghavan, 1998; Pulsford *et al*., 1992; Blazer *et al*., 1987; Brown and George, 1985). However there are several conflicting results that exists regarding the occurrence of MMC in spleen, kidney and liver of fishes Pulsford *et al.* (1992), Kranz and Gercken (1987), Kranz and Peters (1984), Haensly *et al.* (1982), Kranz (1989), Payne and Fancy (1989). Decrease in MMC frequency indicates low level of pollution (Kranz, 1989), on at high levels of pollution the chemotactic and phagocytic activity of macrophage is reduced to decrease in MMC (Weeks and Warnier, 1986). The increase in MMC may be due to involvement of MMC in detoxification processes (Herraez and Zapata, 1991) and involvement in innate and adaptive immunity (Wolke,1992).

In the present study two pigments hemosiderin and melanin are present in the MMC of liver, spleen and kidney and lipofuscin is absent. But, according to Herraez and Zapata (1991), they

found more number of MMC with lipofuschin than hemosiderin and melanin is present only in the kidney. In neotropical fish *Hoplias malabricus* Rabitto *et al*. (2005), hemosiderin and ceroids found in liver and kidney MMC with melanin. The synthesis of melanin and their role in phagocytosis mechanism in liver and kidney is not clear (Mani *et al*., 2001). Phagocytosis is a complex mechanism of hypersensitivity with successive stages including chemotaxis, attachment, ingestion and intracellular digestion (Bols *et al*., 2001). Bols *et al*. (2001) found that cadmium appeared to enhance chemotaxis of peripheral macrophages. Agius and Roberts (2003) suggested that the increase in size, frequency and pigments variation of MMC in conditions of environmental stress as a reliable bimarkers for water quality in terms of both deoxygenation and intragenic chemical pollution.

The present study concludes that the occurrence and changes in MMC and appearance of pigments in MMC of spleen, kidney and liver indicates that the MMC could be considered as a biomarker of stress induced by the various pollutants or toxicants which are present in the aquatic environment.

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